

Claims

1. A method for obtaining fibrinogen from milk, comprising:

5 (a) contacting the milk with a cation exchange chromatography substrate under conditions where the fibrinogen binds to the substrate;

(b) optionally washing the substrate to remove unbound components; and

10 (c) removing the bound fibrinogen from the substrate by using irrigating means, which irrigating means has an increased ionic strength or increased pH or both relative to the conditions in step (a).

15 2. A method as claimed in claim 1 wherein the obtained fibrinogen is at least 60% pure.

3. A method as claimed in claim 1 or claim 2 wherein the condition in step (a) is that the substrate and the milk is at a pH which is higher than the pI value of fibrinogen.

4. A method as claimed in claims 1, 2 or 3 wherein the condition in step (a) is that the substrate and the milk is at a pH which is greater than pH 5.5.

5. A method as claimed in claim 4 wherein the pH is around pH 6.0.

25 6. A method as claimed in any of claims 1 to 5 wherein steps (b) and (c) are performed at a pH greater than pH 5.5 but less than pH 14.0.

7. A method as claimed in claim 6 wherein the washing in step (b) is performed using an irrigating means which has an ionic strength of 0-0.15M and a pH of 5.5-6.5, or an ionic strength of 0-0.1M and a pH of greater than 6.5.

5 8. A method as claimed in claim 6 or claim 7 wherein the irrigating means in step (c) has an ionic strength of equal to or greater than 0.10M and a pH of 5.5-6.5, or an ionic strength of equal to or greater than 0.05M and a pH of greater than 6.5.

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10 9. A method as claimed in any of claims 1 to 8 wherein the milk is whole milk, skimmed milk, milk whey or milk fraction.

10. A method as claimed in any of claims 1 to 9 wherein the milk contains one or more agents capable of disrupting casein micelles.

15 11. A method as claimed in claim 10 wherein the agent is a chelating agent.

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20 12. A method as claimed in claim 10 or 11 wherein the agent is EDTA, EGTA or citrate.

13. A method as claimed in any of claims 1 to 12 wherein the substrate is in a batch format or a column format.

14. A method as claimed in claim 13 wherein the column mode of contacting is by fixed bed adsorption, expanded bed adsorption or fluidised bed adsorption.

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25 15. A method as claimed in any of claims 1 to 14 wherein the fibrinogen is transgenic fibrinogen.

16. A method as claimed in any of claims 1 to 15 wherein the fibrinogen is human fibrinogen.
17. The use of cation exchange chromatography for obtaining fibrinogen from milk.
18. The use as claimed in claim 17 wherein the obtained fibrinogen is at least 60% pure.
19. The use as claimed in claims 17 or 18 wherein the milk contains one or more agents capable of disrupting casein micelles.
20. The use as claimed in claim 19 wherein the agent is a chelating agent.
21. The use as claimed in claim 19 or 20 wherein the agent is EDTA, EGTA or citrate.
22. The use as claimed in any of claims 17 to 21 wherein the cation exchange chromatography is in a batch format or a column format.
23. The use as claimed in claim 22 wherein the column mode of contacting is by fixed bed adsorption, expanded bed adsorption or fluidised bed adsorption.
24. The use as claimed in any of claims 17 to 23 wherein the fibrinogen is transgenic fibrinogen.
25. The use as claimed in any of claims 17 to 24 wherein the fibrinogen is human fibrinogen.

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26. Fibrinogen which is substantially free from viral contamination.

27. Fibrinogen obtainable according to the ~~method~~ as claimed in claims 1 to 16.

5 28. A fibrin adhesive or sealent containing fibrinogen as claimed in claim 26 or 27. - 1

29. A fibrin adhesive or sealent as claimed in claim 28 which contains thrombin, Ca^{2+} 2
and Factor XIII.

10 30. A fibrin adhesive or sealent as ~~claimed~~ in claim 28 or 29 comprising two
components, one component containing fibrinogen and Factor XIII and the other
component containing thrombin and Ca^{2+} .

15 31. A kit comprising fibrinogen as claimed in claim 26 or 27 and instructions for use.

32. A kit as claimed in claim 31 which also comprises thrombin, Ca^{2+} and Factor XIII.

20 33. A method for producing a fibrin adhesive or sealent, comprising mixing
fibrinogen with thrombin, wherein the fibrinogen is as claimed in claim 26 or 27.

34. A method as claimed in claim 33, wherein Factor XIII and Ca^{2+} are mixed with the
fibrinogen and thrombin.

25 35. Fibrinogen as claimed ~~in~~ claim 26 or 27 for use in medicine.

36. A method of surgery or therapy comprising placing on or within an animal or a
body part of an animal a seal or an adhesive, comprising fibrinogen as claimed in
claim 26 or 27.

37. A method as claimed in claim 36 wherein Factor XIII, thrombin and Ca^{2+} are mixed with the fibrinogen before use.

5 38. A method as claimed in claim 36 or 37 wherein the animal is a human.

39. The use of fibrinogen as claimed in claim 26 or 27 for the manufacture of a fibrin adhesive or sealant.

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$\frac{d}{dt} \int_{\Omega} u^m dx = - \int_{\Omega} u^{m+1} dx$ $\frac{d}{dt} \int_{\Omega} v^n dy = - \int_{\Omega} v^{n+1} dy$